

PRISMA Protocol: Biochar Effects on DNRA and Denitrification

Systematic Review and Meta-Analysis Protocol

Registration: PROSPERO (to be submitted)

Date: February 2025

Review Question: How does biochar amendment affect the partitioning of nitrate reduction between dissimilatory nitrate reduction to ammonium (DNRA) and denitrification in soils?

1. PICO Framework

Element	Definition
Population	Soil systems (agricultural, forest, grassland, wetland soils)
Intervention	Biochar amendment (any feedstock, pyrolysis temperature, application rate)
Comparison	Unamended control soils (same conditions without biochar)
Outcomes	Primary: DNRA rates, denitrification rates, nitrate reduction partitioning ratio; Secondary: Functional gene abundances (nrfA, nirS, nirK, nosZ), N2O emissions

2. Search Strategy

2.1 Databases

- Web of Science Core Collection (SCI-EXPANDED, SSCI, CPCI-S)
- Scopus
- Google Scholar (first 200 results)
- China National Knowledge Infrastructure (CNKI)
- PubMed (for microbial ecology studies)

2.2 Search Terms

Concept 1: Biochar

("biochar" OR "bio-char" OR "charcoal" OR "black carbon" OR "pyrogenic carbon" OR "biomass-derived black carbon")

Concept 2: DNRA

("dissimilatory nitrate reduction to ammonium" OR "DNRA" OR "nrfA" OR "nitrite ammonification" OR "nitrate ammonification")

Concept 3: Denitrification

("denitrification" OR "nirS" OR "nirK" OR "nosZ" OR "nitrate reductase" OR "nitrite reductase" OR "N2O reductase" OR "nitrous oxide emission")

Combined Search String (Web of Science):

```
TS=(( "biochar" OR "bio-char" OR "charcoal" OR "black carbon" OR "pyrogenic carbon")
AND
("dissimilatory nitrate reduction to ammonium" OR "DNRA" OR "nrfA" OR "nitrite ammonification" OR
↪ "denitrification" OR "nirS" OR "nirK" OR "nosZ" OR "nitrous oxide" OR "N2O emission"))
```

2.3 Search Limits

- **Publication date:** 2000-01-01 to 2024-12-31
- **Language:** English, Chinese
- **Document type:** Article, Review (for reference mining)

2.4 Additional Search Methods

- Screening reference lists of included studies
- Screening reference lists of relevant reviews
- Forward citation tracking (using Google Scholar)
- Contacting authors for unpublished data

3. Study Selection Criteria

3.1 Inclusion Criteria

Must meet **ALL** of the following:

1. **Study design:** Original research articles published in peer-reviewed journals
2. **Experimental conditions:**
 - Laboratory incubation experiments OR
 - Field experiments with control treatments
3. **Treatment:** Biochar amendment to soil
4. **Control:** Unamended soil under identical conditions
5. **Outcomes:** At least ONE of the following measured quantitatively:
 - DNRA rates (potential or in-situ)
 - Denitrification rates (potential or in-situ)
 - Functional gene abundances (nrfA, nirS, nirK, nosZ) via qPCR
 - N2O emissions (flux or cumulative)
 - 15N tracer studies reporting DNRA/denitrification partitioning
6. **Data availability:** Mean values and measures of variability (SD, SE, or CI) reported or calculable, with sample sizes

3.2 Exclusion Criteria

Exclude if **ANY** of the following apply:

1. Non-peer-reviewed sources (conference abstracts, theses, reports, book chapters)
2. Review articles, meta-analyses, commentaries, editorials
3. Studies without appropriate control treatments
4. Studies reporting only qualitative results
5. Pure culture or enrichment studies (non-soil systems)

- 6. Studies with co-amendments that confound biochar effects (e.g., biochar + fertilizer without separate biochar-only treatment)
- 7. Studies with missing or insufficient statistical information
- 8. Duplicate publications (use most comprehensive/complete data)

4. Study Selection Process

4.1 Screening Stages

Stage 1: Title and Abstract Screening

- Two independent reviewers screen all records
- Use Rayyan QCRI or Covidence for blinded screening
- Resolve conflicts through discussion or third reviewer

Stage 2: Full-Text Screening

- Retrieve full texts of potentially eligible studies
- Two independent reviewers assess eligibility
- Document reasons for exclusion

4.2 Inter-rater Agreement

- Calculate Cohen’s kappa for both stages
- Target: 0.80 (substantial agreement)
- Disagreements resolved by consensus or third reviewer

5. Data Extraction

5.1 Study Characteristics

Category	Variables to Extract
Bibliographic	First author, year, journal, DOI
Study design	Experiment type (field/lab), duration, location
Soil properties	pH, SOC, TN, C/N ratio, texture (sand/silt/clay %), bulk density, land use
Climate	MAT, MAP (from study or WorldClim if not reported)
Biochar properties	Feedstock, pyrolysis temperature, pH, C content, N content, C/N ratio, ash content, surface area, application rate
Experimental conditions	Temperature, moisture, incubation time, N fertilization

5.2 Outcome Data

For each treatment-control pair, extract:

Outcome	Variables
DNRA rate	Mean, SD/SE, n, units (mg N kg ⁻¹ d ⁻¹ or g N g ⁻¹ h ⁻¹)
Denitrification rate	Mean, SD/SE, n, units
N₂O emission	Mean, SD/SE, n, units
nrfA abundance	Mean, SD/SE, n, units (copies g ⁻¹ soil)
nirS abundance	Mean, SD/SE, n, units
nirK abundance	Mean, SD/SE, n, units
nosZ abundance	Mean, SD/SE, n, units

5.3 Data Extraction Rules

1. **Multiple time points:** Extract final measurement (longest duration)
2. **Multiple application rates:** Extract each rate as separate observation
3. **Multiple biochar types:** Extract each type as separate observation
4. **Multiple soil types:** Extract each soil as separate observation
5. **Unit conversion:** Standardize to common units during extraction
6. **Missing variability:** Contact authors; if unavailable, use average CV from similar studies

6. Risk of Bias Assessment

Use a modified version of the Cochrane Risk of Bias tool adapted for ecological studies:

Domain	Assessment Criteria
Randomization	Was allocation to treatments randomized?
Replication	Were there sufficient replicates (n ≥ 3)?
Confounding	Were potential confounders controlled?
Outcome measurement	Were outcomes measured using validated methods?
Selective reporting	Were all expected outcomes reported?

Rate each domain as: Low risk / Unclear / High risk

7. Data Synthesis and Analysis

7.1 Effect Size Calculation

Primary metric: Natural logarithm of response ratio (lnRR)

$$\ln\text{RR} = \ln(\bar{X}_{\text{treatment}} / \bar{X}_{\text{control}}) = \ln(\bar{X}_{\text{treatment}}) - \ln(\bar{X}_{\text{control}})$$

Variance:

$$V\ln\text{RR} = \text{SD}^2_{\text{treatment}} / (n_{\text{treatment}} \times \bar{X}^2_{\text{treatment}}) + \text{SD}^2_{\text{control}} / (n_{\text{control}} \times \bar{X}^2_{\text{control}})$$

7.2 Statistical Model

Three-level random-effects meta-analysis:

- Level 1: Sampling variance
- Level 2: Within-study variance (multiple observations per study)
- Level 3: Between-study variance

Model specification:

$$y_{ij} = \mu + u_i + u_j(i) + \varepsilon_{ij}$$

7.3 Heterogeneity Assessment

- I^2 statistic (total, Level 2, Level 3)
- Q-test for heterogeneity
- Prediction intervals

7.4 Publication Bias

- Funnel plots
- Egger's regression test
- Rosenberg's fail-safe number
- Trim-and-fill method (if bias detected)

7.5 Moderator Analyses

Categorical moderators:

- Soil pH class (acidic < 6.0, neutral 6.0-7.5, alkaline > 7.5)
- Soil texture (sand, loam, clay)
- Land use (cropland, paddy, forest, grassland)
- Biochar feedstock (wood, crop residue, manure, other)
- Pyrolysis temperature (low < 400°C, medium 400-600°C, high > 600°C)
- Application rate (low < 10 t/ha, medium 10-30 t/ha, high > 30 t/ha)

Continuous moderators:

- Soil pH
- Soil organic C
- MAT, MAP
- Biochar C/N ratio
- Application rate

7.6 Sensitivity Analyses

1. Exclude high-risk-of-bias studies
2. Exclude outliers ($|\ln RR| > 3$)
3. Exclude studies with imputed SD

4. Alternative effect size metric (Hedges' g)

8. Software and Packages

- **Screening:** Rayyan QCRI or Covidence
- **Data extraction:** Microsoft Excel or Google Sheets
- **Statistical analysis:** R (v4.3.0+)
 - metafor (v4.0.0+) - main meta-analysis
 - ggplot2 (v3.4.0+) - visualization
 - orchaRd (v1.0.0+) - orchard plots
 - clubSandwich (v0.5.0+) - robust variance estimation

9. Timeline

Phase	Duration	Deliverable
Protocol finalization	1 week	PROSPERO registration
Literature search	1 week	EndNote library
Title/abstract screening	2 weeks	Screened records
Full-text screening	2 weeks	Included studies list
Data extraction	4 weeks	Completed extraction sheet
Statistical analysis	2 weeks	Analysis outputs
Manuscript writing	2 weeks	First draft
Revision and submission	2 weeks	Submitted manuscript

Total estimated time: 16 weeks (4 months)

10. References

Page MJ, et al. (2021). The PRISMA 2020 statement. *BMJ*, 372:n71.

Haddaway NR, et al. (2018). ROSES RepOrting standards for Systematic Evidence Syntheses. *Environmental Evidence*, 7:7.

Protocol Version: 1.0
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